A STUDY OF GRANULOCYTIC ADHERENCE UNDER DIFFERENT ANAESTHETICS

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FOR DOCTOR OF MEDICINE

(ANAESTHESIOLOGY)



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C E R T I F I C A T E

This is to certify that the work of Dr. RAM PAL SINGH, on "A STUDY OF GRANULOCYTIC ADHERENCE UNDER DIFFERENT ANAESTHETICS", which is being presented by him for M.D. (Anaesthesiology) examination, 1986, has been carried out in the Department of Anaesthesiology.

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C O N T E N T S

	PAGE NO.
INTRODUCTION	1 - 3
REVIEW OF LITERATURE	4 - 24
MATERIAL AND METHODS	25 - 32
OBSERVATIONS AND RESULTS	33 - 51
DISCUSSION	52 - 67
SUMMARY AND CONCLUSIONS	68 - 71
BIBLIOGRAPHY	I - XVI
SUMMARY	(In separate cover)

INTRODUCTION

INTRODUCTION

The development of anaesthesia since its introduction in 1846 (16th October 1846 by W.T.G. Morton of Boston) has been erratic, long periods of stagnation being occasionally broken by improvements and advances.

The immunological and carcinogenic side effects of anaesthetics, provide, a long and controversial story in the annals of anaesthesia. Despite frequent lack of agreement about the exact nature and extent of these side effects, they continue to be fascinating and important because they may affect profoundly, the well being of patients by altering their susceptibility to post-operative infections, adverse drug reaction, rejection of transplanted grafts or organs and resistance to tumour. In addition, anaesthesiologists and other persons working in operation theatre may also be affected by virtue of their occupational chronic low exposure.

Immune-responses seem to be depressed after surgical procedure. There is substantial evidence starting from the early observation that prolonged

exposure to nitrous oxide results in leucopaenia in humans and that nearly every anaesthetic depresses white cell production. Since it is well known fact that all anaesthetics are cellular poisons, majority of granulocytic functions are disturbed in persons exposed to anaesthetics. Phagocytic inhibition, in humans and rabbits, after ether anaesthesia is well known since very beginning (Rubin, 1907). Chemotaxis is also known to be depressed after anaesthetics exposure (Moudgil, 1977, Hill et al, 1976). Cells exposed to halothane and nitrous oxide show immunological depression (Bandon, 1977).

The influence of anaesthetics on immune response is of great importance. Both infections and malignancies can be expected to be more devasting in animals or patients who have reduced immuno-competency. Much work has been done on different granulocytic functions like phagocytosis, chemotaxis, but adherence of granulocyte to the endothelium of vessels, is neglected or least studied function. A decrease in granulocytic adherence has been reported in diseases which are associated with increased risk of infections like chronic myeloid leukaemia, diabetes mellitus, acute myeloid leukaemia, multiple myeloma, macro-globulinemia and paroxysmal nocturnal haemoglobinuria (Rabinowitz, 1965; Penny and Galton,

1966 and MacGregor et al, 1978). When all the functions of granulocytes seem to be depressed after exposure of anaesthetics it is very likely that granulocytic adherence may not be an exception.

Considering the significance of the impairment in adherence of granulocytes, as an important factor responsible for recurrent infections in patients during post-operative course and the available literature being very meagre, this study was undertaken..

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The possibility of participation of leucocytes in local inflammatory response was described by Hunter in 1794 and Dutrochet in 1828 (Senn and Jungi, 1975). The haematogenous origin of inflammatory cells was not settled until the late 19th century when Hering (1867) and Cohnheim (1867) demonstrated the haematogenous origin of inflammatory cells in the mesentery of the living frog. Later Liberkuhn (1870) described peri-vascular locomotion of leucocytes on a glass surface. Phagocytosis and chemotaxis were discovered subsequently (Leber, 1888; Gabritschevsky, 1890 and Metchnikoff, 1893).

When microbes invade the skin and mucous membrane they first encounter host antagonists i.e. anti-bacterial antibodies, complement components, the properdin system, the kinin system, lysozymes transferrin and vaso-active neurohormones. These factors reactivate and stimulate the phagocytic cellular response and this humoral cellular interaction usually provides a remarkable host defence against bacterial diseases (Hirsch, 1965). Polymorphonuclear

cells, called microphagocytes by Metchnikoff (1893) form first line of cellular defence of the mammalians.

Phagocytic cells of the body, which are essential for host defence against infection can be divided into -

I. Peripheral blood

1. Polymorpho-nuclear neutrophil,
2. Bilobed eosinophil,
3. Monocyte.

II. Reticulo-endothelial system

1. Alveolar macrophages of lung,
2. Kupffer cells of liver,
3. Spleen and bone marrow macrophages,
4. Other tissue macrophages.

The polymorphonuclear neutrophil is the chief phagocytic cell of blood and can quickly leave the vascular tree by virtue of it's amoeboid movements to engulf and destroy bacteria and other microbes. The bilobed eosinophil, although some what more limited in its phagocytic activity, can also kill pyogenic bacteria. In contrast to the neutrophil and eosinophil, which are end stage cells, the monocyte can transform in the tissues into larger macrophages with broader phagocytic and biosynthetic capacities.

SPECIFIC IMMUNITY

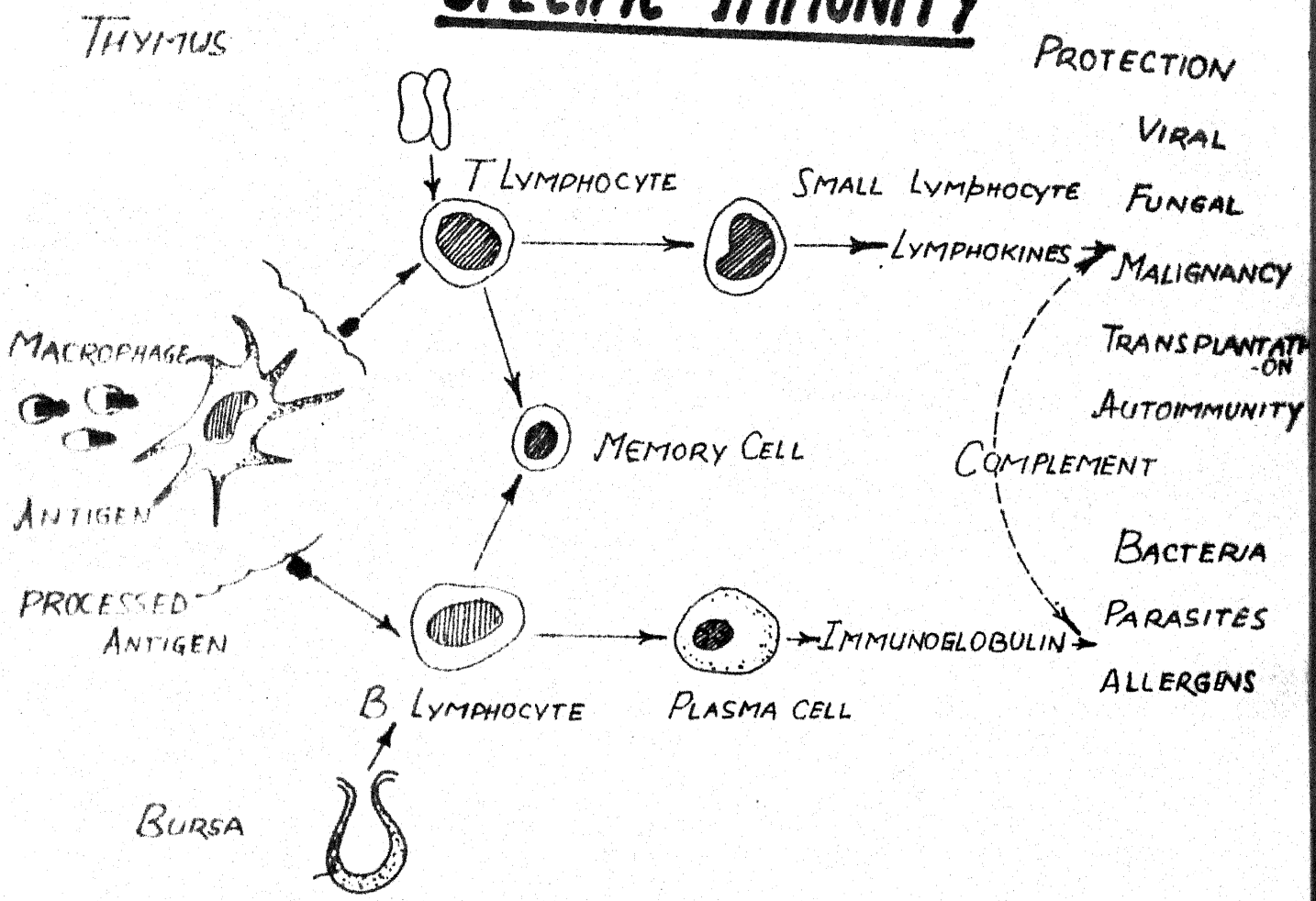


FIGURE - 1 SCHEMATIC REPRESENTATION OF SPECEFIC IMMUNITY

ANTIGENIC MATERIAL IS RECOGNIZED, PHAGOCYTIZED, AND PROCESSED BY MACROPHAGES. INFORMATION IS THEN TRANSFERRED TO CIRCULATING T- LYMPHOCYTES OR B- LYMPHOCYTES, WHICH GIVE RISE EITHER TO SMALL LYMPHOCYTE OR PLASMA CELL. SMALL LYMPHOCYTES PRODUCE LYMPHOKINES OR CLEMICAL MEDIATORS IMPORTANT IN HOST-DEFENSE AGAINST VIRUS, FUNGUS, AND MALIGNANCY. PLASMA CELLS GIVE RISE TO IMMUNO GLOBULINS, WHICH ARE IMPORTANT IN HOST RESISTANCE TO BACTERIA, PARASITES AND OTHER ALLERGENS.

The macrophages of the lung, liver, spleen, skin and bone marrow are part of the reticulo-endothelial system (Baehner, 1975a).

Lymphocytes represent the specific recognising segment of immune response while neutrophil and monocytic macrophages are the non-specific cellular elements that perform the actual destruction of the foreign material.

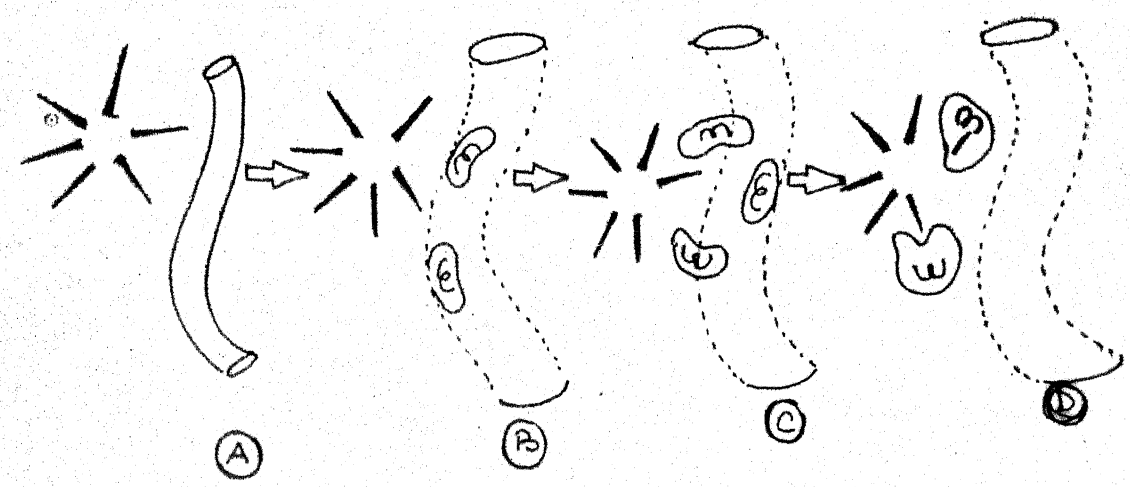
Neutrophils and monocytic macrophages represent an important biological amplification mechanism, central to many inflammatory responses. This system requires few specific recognizing cells like lymphocytes and allows the small number of lymphocytes to signal an immense number of non-specific cells like polymorphonuclear leucocytes and mono-nuclear leucocytes to be activated (Miller, 1975 and Stossel, 1975).

Physiological Mechanism of Phagocytosis (Weston, 1976)

The phagocytic function has been separated into a series of six physiologic stages -

1) Random movement (Non-directional movement)
2) Chemotaxis (Directional movement)
3) Adherence (Attachment to the material)
4) Phagocytosis (Ingestion)

NON-SPECIFIC IMMUNITY



INJURY

VASCULAR RES- PONCE, LEUCOCYTE ADHERENCE

CELLULAR INFILTRATION

PHAGOCYTOSIS BACTERIAL KILLING

FIGURE -2 THE STEPS IN THE INFLAMMATION RESPONSE.

TISSUE INJURY OR BACTERIAL INVASION STIMULATES VASCULAR DILATATION AND INCREASED VESSEL PERMEABILITY, AIDING THE DELIVERY OF PLASMA PROTEINS, ANTIBODIES, AND CIRCULATING LEUKOCYTES TO THE AREA. LEUKOCYTES ADHERE TO THE VESSEL WALLS, MIGRATE BETWEEN THE ENDOTHELIAL CELLS AND ENTER THE INTERSTITIAL SPACE, WITH THE AID OF OPSONINS FROM THE PLASMA PROTEINS, BACTERIA AND CELLULAR DEBRIS ARE PHAGOCYTOSED AND DESTROYED.

5) Activation of the phagocyte

6) Microbicidal activity (Destruction of foreign material).

Random Movement -

Random movement has been defined as non-directional movement of cells. It is measured by the migration of cells through a capillary tube (Random tube migration) or through a porous filter (Random filter migration). It is evident that this non-directional movement is a separate phenomenon from directional movement (chemotaxis) by the observation that in a number of disease states like Chediak-Higashi Syndrome, Diabetes mellitus and Candidiasis with deficient cell mediated immunity, the random movement is normal whereas chemotaxis is abnormal (Miller et al, 1971; Miller, 1973 & 1975 and Clark & Kimball, 1971). Further, capillary tube migration and filter migration may measure separate events as in patients having Chediak-Higashi Syndrome showed defective random filter migration but normal random tube migration (Clark & Kimball, 1971).

Chemotaxis -

This term refers to directional movement of polymorphonuclear leucocytes or mononuclear leucocytes to the sites of microbial invasion. There are two

major steps involved in it. Activation of phagocytic cell membrane, chemotactant and the cell movement. The measurement of chemotaxis is performed with some modification of Boyden chamber (Boyden, 1962). The number of leucocytes that pores through the membrane in response to the stimulus is measured and serve as an index of the tested leucocytes intrinsic motility and tested attractants chemotactic effect. The complement system, which activates chemotaxis, includes trimolicular complex C^5, C^6, C^7 and low molicular weight fragments C^3A and C^5A. Inhibitors of chemotaxis have been found in the serum of few children with recurrent infection (Ward and Schlogel, 1969). Defective chemotaxis due to intrinsic deficiency in neutrophil has been described in patients with diabetes mellitus (Mewat and Baun, 1971). Moudgil and co-workers (1977) using in vitro exposure of polymorphonuclear leucocytes to anaesthetics including lidocaine and marcaine demonstrated retarded chemotaxis in a dose-related fashion.

Adherence -

It is an important phenomenon comprising of sticking of granulocytes to the vascular endothelium near the inflammatory stimulus (Grant, 1974) and also adherence of micro organisms to phagocyte surface which enhances their engulfment. It is called

opsonisation. These processes are facilitated by humoral factors called opsonins (Weston, 1976). Granulocytic margination is the first event to occur after application of stimulus and is an antecedent step to chemotaxis, opsonisation, phagocytosis and microbial killing. The important factors involved in this process are sub-classes of immunoglobulin G (IgG), antibodies and the complement components C_5a, a potent chemotaxin (Ward and Newman, 1969), and C_3b, which is necessary for opsonisation of microbes (Alper et al, 1972). They are recognised by surface receptors on granulocytes and help in making the firm contact with microbes (Cline and Golde, 1977).

Phagocytosis -

After the offending micro-organism is opsonised and contacts the cell surfaces, pseudopodia are extended and the organism is engulfed. The process of phagocytosis is more complex. The technique for assay of phagocytosis involves incubation of a microscopically visible particles with phagocytic leucocytes by using Nitro blue tetra-zolium test (Park et al, 1968). Radio-activity labelled bacteria, starch particles, immune complexes, or erythrocytes and polyvinyl-toluene particles have been utilised to quantitate phagocytosis. The method adopted by Stossel (1975) in which paraffin oil particles

containing the dye oil red O is most widely used. Investigations of the process of ingestion have uncovered many facts of this complex series of events; the importance of cell membrane receptors for IgG and C_3 in the process of opsonisation, the role of micro-tubules and the contractile proteins, actin and myosin within the cell, the importance of the divalent cations, manganese, cobalt, magnesium and calcium in phagocytosis (Weston, 1976).

Activation of the Phagocyte -

After phagocytosis, a series of metabolic events occur that have been called the "respiratory burst". This consists of an increase in glucose utilisation through the hexose monophosphate shunt, an uptake of oxygen, and the production and release of substances, super-oxide (O_2^-) and hydrogen peroxide, which are thought to be important in microbicidal activity (Klebanoff, 1975b). The enzymes responsible for the respiratory burst include NADH and NADPH. These enzymes reduce oxygen to peroxide and reduce colourless nitro-blue tetrazolium dye to a blue compound, formazan that can be quantitated by spectrophotometeric reading (Baehner et al, 1975).

Microbicidal activity -

Following the ingestion of micro-organisms a series of distinct events occur within the polymorphonuclear leucocytes, which lead ultimately to the destruction of the invading organism. The killing and digestion of the ingested material by phagocytic leucocyte is either oxygen dependent or oxygen independent. It occurs with the help of intraphagocytic leucocytic digestive enzymes (Klebanoff, 1975a).

ASSAY METHODS FOR GRANULOCYTIC ADHERENCE -

Different methods for separating leucocytes from erythrocytes and lymphocytes from granulocytes by applying principles of differential densities centrifugation techniques, were developed by using serum albumin (Vallee et al, 1947), gum acacia (Spear, 1948), exchange resin (Tullis, 1952), two albumin solutions of differing densities (Agranoff et al, 1954).

Ottensen (1955) separated lymphocytes from granulocytes and erythrocytes by centrifuging blood in a tube which contains a specially derived perspex body in the capillary tube in it's axis and by adjusting its specific gravity.

Jago (1956) used heparinised blood to separate granulocytes from lymphocytes by using the centrifuging and sendimentation principles. Philippu (1956) used a method described by Szilard (1926) and separated different types of leucocytes by centrifugation of leucocyte suspension in Wintrobe tube.

Ventzke et al (1959) separated leucocytes by method of Skoog and Beck (1956) and then by using plastic tube, separated granulocytes from lymphocytes by centrifugation and sedimentation. Johnson and Garvin (1959) removed granulocytes from heparinised whole blood by passage through a short column of siliconised glass wool, which adhere to it, while lymphocytes were yielded in the effluent blood.

Garvin (1961) further intensively studied varieties of factors affecting leucocytes and platelet adherence to siliconized glass beads column instead of glass wool.

Rabinowitz (1964) used glass beads column, described by Garvin (1961), to separate various types of leucocytes. He again (1965) used same method to study adherence and separation of leucaemic cells on glass beads column.

Brandt (1965), also assayed granulocytic adherence by taking siliconised glass beads 0.1 mm in diameter, which were packed to a height of 3 c.m. over small pieces of glass wool, in a siliconised glass syringe 1 c.m. in diameter, the whole, being enclosed in a water jacket at 37°C. 2 ml of heparinised whole blood (30 units/ml) was added to the column and after 10 minutes allowed to flow out from the column. The flow rate was adjusted by controlling the pressure on the plunger of the syringe to 0.1 ml/ minutes. An absolute neutrophil count (TLC x % of neutrophil) was performed over the influent and effluent blood and ratio was termed as "Adhesiveness index" (A.I).

Kvarstein (1969) also used glass beads column to study granulocytic adherence. Penny et al (1966) used method devised by Brandt (1965), to see the effect of various drugs and physical factors on granulocytic adherence.

Bryant and Sutcliffe (1972), prepared leucocyte rich plasma from heparinised blood by centrifuging. The granulocytic adherence to glass was determined by using capillary glass tubes and adhesion was expressed as percentage of control adhesion or as percentage of test W.B.C. count.

$$\text{Percentage of control adhesion} = \frac{\text{Test A.I.}}{\text{Control A.I}} \times 100$$

$$\text{Percentage of W.B.C. count} = \frac{\text{Test A.I}}{\text{Test W.B.C. count}} \times 100$$

This method was advantageous over previous techniques (Garvin, 1961). In contrast to procedures employing glass bead columns, only adherent cells are measured and artificial evaluation of adhesion due to sequestration of cells can be excluded. Furthermore, cells in capillary tubes can be examined directly with a microscope and differential count and characteristics such as cell aggregation can be noted.

Fenn (1923), Garvin (1961) and Kvarstein (1969) noted that optimal adhesion occurs at 37°C and adhesiveness is reduced as the temperature is lowered. Bryant and Sutcliffe (1972) also observed similar findings. Bryant and Sutcliffe (1973) saw effect of cyclic AMP on granulocytic adherence by using glass beads column.

Mac Gregor et al (1974) described a new and simple assay method using pasteur pipettes and nylon fibres instead of glass beads. Since then, this method has been used by Lentnek et al (1976), Bagdade et al (1978), Mac Gregor et al (1978).

Boxer et al (1978). Klempner and Gallin (1978) used nylon wool and tuberculin syringes with 27 gauge needles in place of pasteur pipettes and leucopac nylon fibres. Schiffer et al (1977) also used nylon fibres to study the effect of local anaesthetic agents on granulocytic adherence. Present study was carried-out with Klempner and Gallin technique having tubercular syringe with nylon wool column upto two centimeter height from below and 21 gauge needle.

Granulocytic adherence and infection -

Adherence is an essential step in the physiology of phagocytosis and host defence. Granulocytes must adhere to the vascular endothelium before diapedesis into extravascular compartment, directional movements, phagocytosis and killing of bacteria at the site of inflammation. Inhibition of any of these steps may impair the host defence against bacterial invasion. Adherence is the least studied aspect of granulocytes uptil now.

Granulocytic adherence has been studied in a few diseases associated with recurrent infections and has found to be associated with impairment of other neutrophilic functions. Rabinowitz (1965) and Penny and Galton (1966 b) observed impaired granulocytic adherence in chronic myeloid leukaemia.

acute myeloid leukaemia, myelomatosis, macroglobulinaemia and paroxysmal noctural haemoglobinuria. Granulocytic adherence to nylon fibres was found to be depressed in 12 out of 29 patients of multiple myeloma (Mac Gregor et al, 1978). Lazy leucocyte syndrome and Chediak Higashi Syndrome are associated with decreased adherence of granulocytes (Boxer et al, 1974, 1976 and 1978).

Inflammation augmented the granulocytic adherence to nylon fibres in vitro. Mean adherence in these patients was twice normal (56.4 ± 5.6% V/s 29.4 ± 5.2%) and was inhibited by in vitro administration of anti-inflammatory agents (Lentnek et al, 1976).

The anti-inflammatory drugs interfering with the granulocytic adherence are corticosteroids, aspirin, ethanol, acetaminophen, indomethacin, phenylbutazone, colchicine, tetracycline, lidocaine, EDTA, normal saline and bradykinins (Mac Gregor et al, 1974, 1976; Penny et al, 1966a; and Schiffer et al, 1977). Ethanol, however in mild to moderate doses (0.2 gm/litre concentration) increases but in high doses (6.4 gm/litre concentration) inhibits granulocytic adherence (Hallengren and Pergreen, 1978). These drugs when mixed with adherence increasing factor of inflammatory diseases, the augmenting effect

of this factor is neutralised and normal granulocytic adherence results (Mac Gregor, 1976). O'Floherty et al (1978) suggested that activated complement induces stickness of granulocytes, which endangers their sequestration. The results help to explain the recently reported pulmonary leucostasis and dysfunctions accompanying haemodialysis (Mac Gregor, 1977). An increase in cyclic AMP level is also known to cause decrease in granulocytic adherence (Brayant and Sutcliffe, 1973).

Penny (1966a) suggested that chelating agent like EDTA binds calcium ions and this calcium can be replaced by other divalent cations like magnesium, manganese and nickel. The contact between the neutrophil surface and glass surface or phagocytosed particle might thus involve a divalent electrophilic bridge. Complement components are required for chemotaxis (Boyden et al, 1965 and Ward et al, 1965) and the reaction of neutrophils to both chemotactic and emigration stimuli would seem to involve at least in part a membrane alteration resulting in increased adhesiveness.

Anaesthetics enhance infection by depression of phagocytosis and inhibition of mobilisation of phagocytes into the area of infection (Bruce & Wingard, 1971). Grahm (1911) observed reduction in

phagocytosis by human and animal leucocytes when exposed in vitro & vivo to different bacteria during ether anaesthesia.

Goldstein et al (1971) observed that cyclopropane and methoxyflurane reduce the murine pulmonary bactericidal activity. Bruce (1976) found tenfold reduction in number of phagocytised bacteria during halothane anaesthesia, in addition number of neutrophil was also decreased in his study. Kosciolek (1967) reported depressed phagocytosis by neutrophilis during ether and halothane anaesthesia in both man and rabbits. Moudgil et al (1977), Stanley et al (1976) and Hill et al (1977) showed that inhalational anaesthetics including halothane depress the chemotaxis of neutrophil with clinically used concentration in human. All these studies suggest that anaesthesia is the prominant cause of depressed phagocytosis which gradually returned to normal some 60 hours later (Bruce & Wingard, 1971). The degree of leucocytic depression is related to duration of anaesthetic exposure (Rubin 1904; Snel, 1903; Wingard, 1971).

A number of studies (Eastwood et al, 1963; Lassen, 1956; Parbrook, 1967) indicate that exposure to an anaesthetic concentration of N_2O may produce

leucopaenia in animals and in men. It was further shown by Kripke et al (1977) that 20% N_2O is the lowest concentration required for producing such leucopaenia.

Cullen et al (1975) showed that phagocytosis is slightly depressed after induction of anaesthesia but before operative intervention and this existed for halothane, N_2O as well as for opioid N_2O anaesthesia, thereby showing that this depression was independent of any particular type of anaesthetic agent (Cascorbi, 1981).

On the other hand the effect of local anaesthetics on phagocytosis are less well studied. Moudgil and co-workers (1977) demonstrated retarted chemotaxis in a dose related fashion by using in vitro exposure of neutrophil to lidocaine and marcaine. This finding was important at the site of local anaesthetic infiltration (Duncan & Cullen, 1977). Stanley et al (1976) extended these findings to in vivo exposure and were able to show that patient receiving lidocaine epidural with epinephrine showed depressed chemotaxis, this effect was reversible after 8 hours. Stanley et al (1978), Hill & Colleagues (1977) further demonstrated this effect in the absence of surgical stress.

ANAESTHESIA AND THE CELL

The cell - the basic unit of life - has a pivotral role in body defences and is susceptible to anaesthetic actions (Moudgil et al, 1976). Heilbrunn (1920) demonstrated that cytoplasm of sea-urchin eggs becomes less viscous after exposure to ether, chloroform, paraldehyde, chloral hydrate and urethane. (Seitriz (1941) further observed that chloroform and cyclopropane stopped cytoplasmic streaming in the slime mould physarum polycephalum by a rapid and reversible gelation of the cytoplasm. This effect was suggested to be due to rapid and reversible locking of the linear protein molecule).

Schoenborn, Watson and Kendrew (1965) and Schoenborn (1968) demonstrated that anaesthetic agents can interact with proteins. The anaesthetic agents may affect the antigen and antibody reactions by altering the configuration of various molicular receptor sites (Moudgil et al, 1976).

Anaesthesia and cell motility -

Nunn et al (1970) demonstrated reversible inhibition of lymphocyte motility on exposure to halothane. Of further interest are the observation that mobilisation of phagocytes is also adversely

affected by anaesthesia. Bruce (1966) observed that halothane anaesthesia caused decreased mobilization of neutrophils in response to intra-peritoneal injection of pseudomonas endotoxin. Kimbell and Brody (1963) observed that focal accumulation of neutrophils in rabbit's ear skin windows, was markedly depressed after anaesthetising the animals with ether or halothane. Lowenberg (1934) also made similar observations of depressed locomotion in vitro.

Anaesthesia and random cell movement -

The spontaneous motilities of mouce leucocytes (Nunn, Sharp and Kimbell, 1970; Rabinovitch and Destefana, 1974) and a variety of unicellular organisms (Bruce, 1975; Sharp et al, 1969; Wiklund et al, 1972) are decreased by halothane, methoxyflurane, cyclopropane, chloroform and ether. The effects are reversible and may be secondary to anaesthetic effects on micro-tubular systems necessary for modification of the membrane structure (Nunn et al, 1970).

Anaesthesia and chemotaxis -

In contrast to random cell motility, the attraction of leucocytes to a nidus of infection is directional and under the influence of chemotactic factors. Although chemotaxis is impaired by ethanol

and thermal injury (Seifter et al, 1973), the effects of anaesthesia and operation have not been ascertained. Bruce (1966) demonstrated reduced leucocyte mobilisation to the mouse peritoneal cavity in response to salmonella or pseudomonas lipopolysaccharide when mice were anaesthetised with 1% halothane, but the influence of altered splanchnic blood flow during anaesthesia could not be excluded.

In human patients, the leucocyte mobilisation viewed through a skin window is not inhibited by surgical procedures during cyclopropane, N_2O and ether anaesthesia (Brayton et al, 1970). However, ethanol, shock and diabetes are capable of inhibiting the response. The effects of anaesthetics on leucocyte mobilisation and directional motility in vitro are unknown (Duncan & Cullen, 1976).

Anaesthesia and cellular adherence -

This is the least studied function in relation to anaesthetics. Leucocyte adherence to the vessel wall is inhibited by topically applied lidocaine in a dose related manner (Giddon et al, 1972). But the effects of other anaesthetics are unknown (Duncan and Cullen, 1976).

Anaesthesia and phagocytosis -

In 1911, Graham showed an inhibition of phagocytosis when human and rabbit leucocyte were exposed to ether. Hamburger (1916) reported a dose related inhibition of phagocytosis by equine leucocytes too in vitro exposure of chloroform. Bruce (1967) and Kosciolek (1967) reported similar results with ether and halothane anaesthesia. Cullen, Hume and Chretien (1972) reported decreased phagocytosis in patients during halothane or nitrous oxide - narcotic anaesthesia without surgery.

Recent studies have demonstrated the presence of IgG receptors on the cell surfaces of monocytes and neutrophils, but Douglas (1970) has suggested that neutrophils require complement in addition to IgG for efficient phagocytosis. Moudgil et al (1976) have speculated that anaesthetic agents hinder opsonization or alter the cell receptor sites, thereby reducing the phagocytosis.

More recent studies using in vitro technique with halothane and N_2O have demonstrated only minimal effects upon phagocytosis by human leucocytes (Cullen, 1974; Cullen et al, 1972). Local anaesthetic inhibition of mouse macrophages (Rabinovitch, 1974)and human leucocytes (Cullen, 1974) is demonstrable only with very high concentrations of drugs which are usually achieved only at the site of infiltration.

Narcotics in clinical use have not been evaluated, however, levarphanol the structural analogue of morphine can cause 80-90% inhibition of phagocytosis in vitro (Wurster et al, 1971, Zucker Franklin et al, 1971).

The administration of 1% halothane impairs phagocytosis of peritoneal salmonella in mice (Bruce, 1967) but human studies after halothane - N_2O, Pentothal Inovar - N_2O, or morphine - d tubocurarine - N_2O anaesthesia (Cullen et al, 1975; Rosenbaum et al, personal communication to Duncan & Cullen, 1976), without operation have shown only minimal impairment of latex particle phagocytosis by peripheral blood leucocytes. The N.B.T. reduction is reduced after morphine - N_2O anaesthesia (Rosenbaum et al, 1976, personal communication) but this may represent a steroid effect rather than a direct effect of the anaesthetic agents (Chretien et al, 1972). Reduced phagocytic activity by fixed macrophages of the reticulo-endothelial system has been demonstrated during anaesthesia in man (Lofstrom et al, 1974) and in animals (Goldstein et al, 1971).

MATERIAL AND METHODS

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The present study of Granulocytic Adherence under different anaesthetics was carried out on 60 patients admitted to different surgical wards of M.L.B. Medical College and Hospital, Jhansi, for their different ailments.

SELECTION OF CASES -

Only the ASA grade I and ASA grade II patients were selected for this study. The subjects studied were related to general surgery, Gynaecological and Obstetrical surgery. All these patients were examined before surgery and grouped at random into 3.

Group I - These patients were given sub-arachnoid block with 5% Heavy Xylocaine.

Group II - These patients were given sub-arachnoid block with 1% Heavy Bupivacaine (Marcaine).

Group III - These patients received general anaesthesia having induction with Thiopentone sodium + Succinylcholine and maintenance with Oxygen/Nitrous oxide and Flaxedil.

Group IV - These patients received general anaesthesia having induction with Thiopentone sodium + Succinylcholine and maintenance with Oxygen/Nitrous oxide and Ether.

Group V - These patients were given general anaesthesia having induction of Thiopentone sodium + Succinylcholine and maintenance with Oxygen/Nitrous oxide and Halothane.

Table I

Showing distribution of cases according to the type of anaesthesia used.

Groups	No. of cases	Type of anaesthesia used
I	10	Sub-arachnoid block with 5% Heavy Xylocaine
II	10	Sub-arachnoid block with 1% Heavy Bupivacaine (Marcaine)
III	15	Thiopentone + Scoline. N_2O/O_2/Flaxedil
IV	12	Thiopentone + Scoline; N_2O/O_2/Ether
V	13	Thiopentone + Scoline; N_2O/O_2/Halothane.
Total	60	

PRE-MEDICATION -

Patients in group I and II were given Inj. Atropine 0.6 mg intra-muscularly 40 minutes before surgery and group III, IV, V received 0.6 mg Atropine with Thiopentone sodium as pre-medication.

Patients having evidence of infection or renal insufficiency were not included in this study. Patients taking drugs, known to alter granulocytic adherence such as Aspirine, Oxyphenbutazone; Corticosteroids; Colchicine, Disodium versenate or Alcohol were also excluded from the present study. Patients having blood transfusion within 24 hours of surgical procedure were also discarded.

On the day of operation and next day blood samples were collected under identical conditions. Blood sample just before the induction of anaesthesia was taken as the control for the same patient. In this way each of the 60 patients served as it's own control before induction of anaesthesia.

Total 4 samples of blood were collected for granulocytic adherence assay. Sample of 3 ml blood were collected in heparinized vial (50 units/ml) just before the induction of anaesthesia (control sample); 30 minutes after induction of anaesthesia; immediately after recovery from anaesthesia and last and 4th sample

24 hours after surgical procedure. The granulocytic adherence was determined by the method described below (Klempner and Gallin, 1978).

ADHERENCE ASSAY -

The method required the following preparations to study the granulocytic adherence under different anaesthetics.

Blood samples of 3 ml were taken by a sterile dry syringe from antecubital veins at different times described earlier, and collected in vials containing Heparin (50 I.U./ml of blood).

TUBERCULIN SYRINGE AND PREPARATION OF NYLON WOOL COLUMN -

Dry and clean tubercular syringes fitted with 21 gauze needles were taken for the procedure.

Seventy milligrams nylon wool fibers (Wendy Brilon) were weighed on physical balance. These fibers were packed from top inside the lumen of tuberculin syringe by a metallic stick, upto a height of 2 centimetre from below it means upto the mark .40 on tuberculin syringe from below. The columns were not flushed or washed with any fluid before use. The column height and gauze number of needles used are critical measurements because increased adherence

FIGURE SHOWING THE APPARATUS USED FOR FILTERATION OF BLOOD

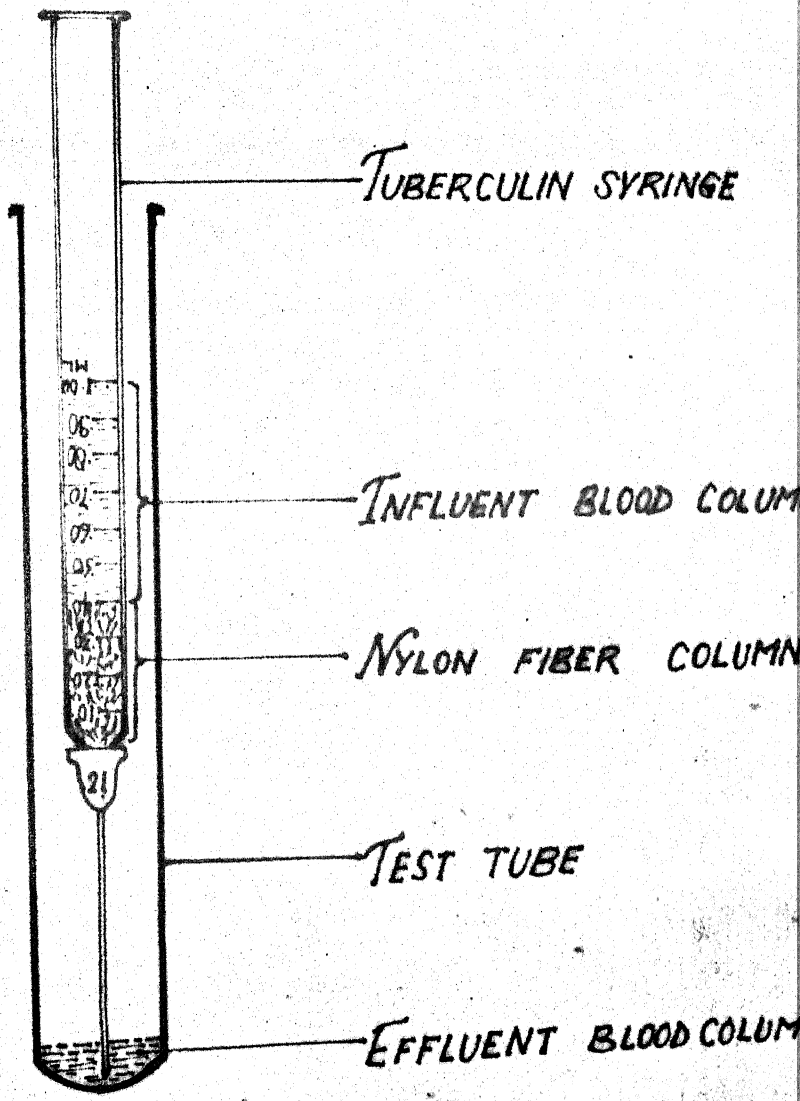


FIG NO. 3

occurs when column is packed more tightly or when the gauze number of needle is larger (aperture is narrower) and vice versa. Two tuberculin syringes were used for each sample and were kept in the test tubes, placed in the test tube racks. The apparatus used is shown in figure 3.

ASSAY METHOD -

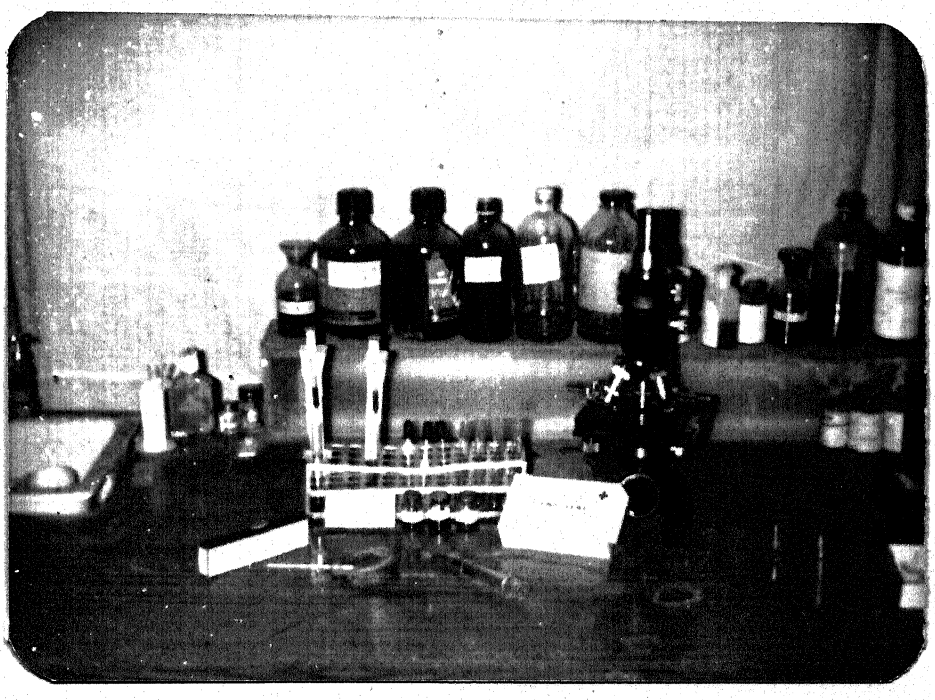
Samples of 3 ml each of heparinised blood (50 I.U./ml of blood) were taken. Total leucocyte count was done by Neubaur chamber and differential count was done by making blood smear and staining with Leishman's stain. Absolute granulocyte was calculated (Total leucocyte count x percentage of granulocytes).

Then one ml of blood was poured by the syringe into the tuberculin syringe kept in the test tube placed vertically in the test tubes rack. The blood was allowed to filter for 10 minutes at room temperature. The gravity and the pressure of the blood column acted as the filtering force. Absolute granulocyte count was calculated again in the similar way from effluent blood. Two tubercular syringes were used for each sample and mean of the granulocyte count was taken for calculation.



Photograph - 1

Showing filteration of blood through nylon wool fibres.



Photograph - 2

Showing appliances used in present study.

The observations and calculations were recorded in the following proforma -

Proforma

Case Serial No. M.R.D. No.

Name : Age / Sex :

Address : Ward/Bed No. :

D.O.A. : Date of operation :

Diagnosis :

Operation done :

Nature of operation : Major / Minor
Routine / Emergency
ASA Grade

Surgeon : Anaesthetist :

Chief complaints :

Personal history : Nutritional status
Vegetarian/Non-vegetarian
Any addiction.

Past history (especially drugs intake) H/of taking Aspirin/Corticosteroid/Tetracycline, Indomethacin, Phenylbutazone & Acetaminophen.

History suggestive of - Diabetes
- Koch's
- Other chronic inflammation

General Examination :

G.C.	Built
Pulse	Respiratory rate
B.P.	Temperature
Pallor	Clubbing

Cyanosis — Oedema
Hydration — Jaundice
Thyroid swelling — Spines
Skin disease
C.V.S.
C.N.S.

- Respiratory system

- Abdomen - Liver — - Kidney
 - Spleen — - Others.
 - Ascitis

Investigations :

- Blood - TLC — DLC - P, L, E, M, B
 Hb% — E.S.R.
- Urine - Albumin
 Sugar
 M/E

Anaesthetic Record -

Pre-medication

Induction - Pentothal - Scoline
OR
- Spinal subarachnoid block.

Maintenance :

I	II	III
N_2O / O_2 / Ether	N_2O / O_2 / Halothane	N_2O/O_2/Relaxant

IV	V
Subarachnoid block with 5% Xylocaine	Subarachnoid block with 2% Marcain

Complications if any :

Transfusion used :

Observation -

		T.L.C. per cu.mm. (a)	Granulocyte(%) (b)	Absolute Granulocyte count (axb)	% of granulocyte adhered
Just before induction	Before filteration				
	After filteration				
30 minutes after induction	Before filteration				
	After filteration				
Immediate after recovery from anaesthesia	Before filteration				
	After filteration				
24 hours after surgical procedure	Before filteration				
	After filteration				

Any other remarks :-

OBSERVATIONS AND RESULTS

OBSERVATIONS AND RESULTS

The present study was carried out on 60 patients having different surgical ailments to study the granulocytic adherence in vitro just before induction, 30 minutes after induction of anaesthesia, just after recovery from anaesthesia and 24 hours after surgical procedure with different anaesthetic agents. Each patient was termed it's own control before induction of anaesthesia.

The subjects studied were selected from various surgical wards (General surgery, Gynaecological and Obstetric surgery) of M.L.B. Medical College and Hospital, Jhansi, U.P. Out of the 60 cases, 23 (38.33%) were of general surgery and 37 (61.67%) were of Gynaec. & Obst. department, who admitted in different wards for different surgical procedures.

None of these patients having any evidence of renal disease, ketoacidosis, history of intake of drugs which impair granulocytic adherence, e.g. aspirin, phenylbutazone, corticosteroids, colchicine, ethanol, indomethacin etc.

Table II

Showing distribution of cases of different types of surgery.

Group of cases	Number of cases			Percentage of cases
	Male	Female	Total	
General Surgery	20	3	23	38.33
Gynaec./Obst. Surgery	-	37	37	61.67
Total	20	40	60	100.00

Only the ASA grade I and ASA grade II patients were selected for this study. Out of the 60 cases, 4 (6.67%) were from ASA grade I and 56 (93.33%) were from ASA grade II.

Table III

Showing distribution of cases according to ASA grading.

Group of cases	Male	Female	Total	Percentage
ASA grade I	3	1	4	6.67
ASA grade II	17	39	56	93.33
Total	20	40	60	100.00

Age and sex

The age and sex distribution of 60 studied patients is given in table IV. Out of 60 patients studied, 20 were males and 40 were females. The mean age for these patients was 31.6 years with the range of 10 to 65 years. The maximum cases were in young adults group (25-34 years) in which 23 patients (38.33%) were studied.

Table IV

Age and sex distribution of the patients.

Age group in years	Male	Female	Total	Percentage
5 - 14	1	-	1	1.67
15 - 24	6	8	14	23.33
25 - 34	3	20	23	38.33
35 - 44	5	8	13	21.67
45 - 54	2	4	6	10.00
55 - 64	2	-	2	3.33
65 - 74	1	-	1	1.67
Total	20	40	60	100.00

Major/Minor surgery

Out of these 60 patients, 36 (60.00%) having major surgical procedures and 24 (40.00%) having minor surgical procedures. Table V shows distribution of cases according to major and minor surgical procedure.

Table V

Showing distribution of cases according to major and minor surgical procedure.

Group	General Surgery		Gynaec. & Obst. surgery	Total cases	Percentage
	Male	Female			
Major procedure	4	2	30	36	60.00
Minor procedure	16	1	7	24	40.00

In the present study patients were grouped at random into 5.

Out of 60 patients, 10 patients (16.67%) in group I were given sub-arachnoid block with 5% Xylocaine heavy. Another 10 patients (16.67%) in group II received sub-arachnoid block with 1% Bupivacaine (Marcaine) heavy. Fifteen patients (25.00%) in group III were induced with Thiopentone sodium + Succinyl choline (Scoline) and maintained with N_2O/O_2/ Flaxedil. Twelve patients (20.00%) in group IV were induced with Thiopentone sodium + Succinyl choline and maintained with N_2O/O_2/Ether and in the last group of 13 patients (21.66%), induction was done by Thiopentone sodium + Succinyl choline and maintained with N_2O/O_2/ Halothane. Table VI shows the distribution of cases according to the anaesthetic agent received during anaesthesia.

Table VI

Showing distribution of cases according to the anaesthetic agents used.

Group	Anaesthetic agents used	Male	Female	Total	Percentage
I	Suberachnoid block with 5% Xylocaine heavy	5	5	10	16.67
II	Suberachnoid block with 1% Bupivacaine (Marcaine) heavy	3	7	10	16.67
III	Thiopentone sodium + Succinyl choline — N_2O/O_2/Flaxedil	4	11	15	25.00
IV	Thiopentone sodium + Succinyl choline — N_2O/O_2/Ether	2	10	12	20.00
V	Thiopentone sodium + Succinyl choline — N_2O/O_2/Halothane	6	7	13	21.66
	Total	20	40	60	100.00

Table VII

Showing percentage of adherence when 10 patients were given 5% Xylocaine heavy as subarachnoid block.

Sl. No.	Name	Age	Sex	PERCENTAGE OF ADHERENCE			
				Sample I (Control samples) Before induction of anaesthesia	Sample II 30 minutes after induction	Sample III After recovery from anaesthesia.	Sample IV 24 hours after surgical procedure
1	A.K.	30	M	88.1	87.3	85.0	87.7
2	R.K.	25	F	78.7	76.4	68.5	76.9
3	M.D.	38	F	87.1	86.4	86.6	85.9
4	V.S.	20	M	86.6	85.2	86.8	86.1
5	P.D.	20	F	89.5	88.2	86.7	87.2
6	K.D.	30	F	90.0	87.9	87.9	88.1
7.	R.P.	38	M	90.1	89.6	89.0	89.7
8	S.S.	55	M	89.5	89.5	87.6	89.7
9	T.R.	45	M	87.3	86.9	86.3	87.3
10	L.D.	33	F	89.2	89.1	88.8	88.8
Mean		33.40		87.66	86.65	85.32	86.74
S.D.		±11.03		±3.41	±3.86	±6.02	±3.69
S.E.		± 3.57		±1.07	±1.22	±1.90	±1.11

S.D. = Standard deviation, S.E. = Standard error.

Table VIII

Showing percentage of adherence when 10 patients were given 1% Duplvecaine (Marcaine) Heavy as subarachnoid block.

Sl. No.	Name	Age	Sex	PERCENTAGE OF ADHERENCE			
				Sample I (Control) Before induction of anaesthesia	Sample II 30 minutes after induction of anaesthesia	Sample III After recovery from anaesthesia	Sample IV 24 hours after surgical procedure.
1	K.D.	40	F	87.5	85.9	85.8	85.0
2	S.D.	38	F	92.2	91.2	90.4	91.4
3	U.D.	22	F	93.4	93.4	92.3	90.3
4	S.D.	35	F	91.2	88.8	87.6	87.4
5	P.D.	30	F	90.9	89.6	89.5	88.4
6	S.B.	45	F	88.6	87.5	87.4	88.1
7	D.D.	20	M	88.7	87.6	87.9	87.9
8	P.D.	40	M	88.4	86.7	87.3	87.9
9	G.K.	42	F	90.3	89.1	87.8	89.2
10	M.P.	65	M	88.2	87.4	87.4	88.1
	Mean	37.7		89.94	88.72	88.34	88.37
	S.D.	±12.71		±1.95	±2.24	±1.87	±1.71
	S.E.	±4.02		±0.61	±0.70	±0.59	±0.54

Table IX

Showing percentage of adherence when 15 patients were induced with Thiopentone sodium + Succinylcholine and maintained with N_2O/O_2/Flaxedil.

Sl. No.	Name	Age	Sex	PERCENTAGE OF ADHERENCE			
				Sample I (Control) Before induction of anaesthesia	Sample II 30 minutes after induction of anaesthesia	Sample III After recovery from anaesthesia	Sample IV 24 hours after surgical procedure
1	S.R.	18	M	89.8	71.0	75.8	83.5
2	P.K.	30	F	90.9	82.8	83.2	86.1
3	K.P.	20	M	86.1	79.9	79.8	80.8
4	B.B.	30	F	93.0	84.9	86.5	87.1
5	M.K.	28	F	94.8	90.2	72.7	81.8
6	A.K.	25	F	93.5	87.3	87.9	89.7
7	G.D.	20	F	90.9	85.9	84.1	88.6
8	U.D.	25	F	89.5	84.9	84.0	85.1
9	M.L.	40	M	92.1	86.8	85.0	89.4
10	A.B.	30	F	88.4	83.3	81.1	85.7
11	S.K.	32	F	90.6	86.6	79.0	86.5
12	B.L.	52	M	92.6	88.8	83.5	89.2
13	M.L.	27	F	91.2	85.2	81.8	87.7
14	D.R.	52	F	91.1	86.7	83.6	86.8
15	P.N.	20	F	91.1	87.1	84.1	87.6
	Mean	29.93		91.04	84.76	82.14	86.37
	S.D.	±10.61		±2.12	±4.54	±3.98	±2.66
	S.E.	±2.74		±0.54	±1.17	±1.02	±0.68

Table X

Showing percentage of adherence when 12 patients were induced with Thiopentone sodium + Succinylcholine and maintained with N_2O/O_2/Ether.

Sl. No.	Name	Age	Sex	PERCENTAGE OF ADHERENCE			
				Sample I (Control) Before induction of anaesthesia	Sample II 30 minutes after induction of anaesthesia	Sample III After recovery from anaesthesia	Sample IV 24 hours after surgical procedure
1	G.S.	18	F	90.6	83.6	87.1	87.7
2	H.K.	30	F	89.1	82.2	80.4	84.9
3	T.D.	28	F	93.3	89.6	88.1	92.8
4	N.S.	26	F	90.4	81.1	80.9	83.4
5	S.J.	45	F	82.6	78.3	78.6	81.8
6	G.D.	40	F	89.1	86.9	88.1	88.2
7	M.S.	30	M	89.2	82.7	83.0	88.9
8	S.K.	35	M	92.7	88.9	87.1	91.7
9	K.D.	25	F	90.8	86.7	85.1	85.4
10	R.K.	35	F	90.1	85.5	84.9	89.4
11	K.D.	23	F	90.7	85.8	87.2	88.8
12	K.D.	45	F	92.9	86.9	88.1	90.3
	Mean	31.66		90.12	84.85	84.88	87.76
	S.D.	±8.55		±2.78	±3.33	±3.37	±3.32
	S.E.	±2.47		±0.80	±0.96	±0.97	±0.95

Table XI

Showing percentage of adherence when 13 patients were induced with Thiopentone sodium + Succinylcholine and maintained with N_2O/O_2/Halothane.

Sl. No.	Name	Age	Sex	PERCENTAGE OF ADHERENCE			
				Sample I (Control) Before induction of anaesthesia	Sample II 30 minutes after induction of anaesthesia	Sample III After recovery from anaesthesia	Sample IV 24 hours after surgical procedure
1	M.S.	28	F	90.0	85.8	85.8	87.7
2	V.D.	33	F	91.4	84.8	84.8	86.4
3	M.D.	16	F	91.6	85.7	85.7	88.3
4	A.K.	10	M	88.2	80.8	79.6	85.7
5	B.W.	25	F	88.7	80.5	80.5	85.3
6	N.K.	15	M	91.1	83.1	80.3	86.6
7	B.R.	35	M	90.1	85.3	85.3	83.9
8	J.P.	60	M	88.1	83.5	82.9	84.2
9	K.D.	25	F	90.7	87.1	80.8	85.9
10	M.D.	20	F	90.8	88.3	79.9	85.3
11	S.R.	32	M	89.8	84.7	79.6	85.5
12	T.R.	22	M	89.6	82.7	81.2	84.5
13	A.R.	35	F	90.4	84.9	84.9	86.9
	Mean	27.38		90.03	84.40	82.40	85.86
	S.D.	±12.62		±1.14	±2.25	±2.53	±1.30
	S.E.	±3.50		±0.31	±0.62	±0.70	±0.36

Table XII A

Showing mean percentage of adherence and standard deviation in different groups.

Groups	Anaesthetic agents used	No. of patients studied	Mean percentage of adherence and standard deviation							
			Sample I (Control)		Sample II		Sample III		Sample IV	
			Mean percentage	Standard deviation	Mean percentage	Standard deviation	Mean percentage	Standard deviation	Mean percentage	Standard deviation
I	Subarachnoid block with 5% Xylocaine	10	87.66	±3.41	86.65	±3.86	85.32	±6.02	86.74	±3.69
II	Subarachnoid block with 1% Marcaine	10	89.94	±1.95	88.72	±2.24	88.34	±1.87	88.37	±1.71
III	Thiopentone + Scoline — N_2O/O_2/Flaxedil	15	91.04	±2.12	84.76	±4.54	82.14	±3.98	86.37	±2.66
IV	Thiopentone + Scoline — N_2O/O_2/Ether	12	90.12	±2.78	84.85	±3.33	84.88	±3.37	87.76	±3.32
V	Thiopentone + Scoline — N_2O/O_2/Halothane	13	90.03	±1.14	84.40	±2.25	82.40	±2.53	85.86	±1.30

Table XII B

Showing statistical significance between samples in different groups.

Group	No. of patients	Statistical significance of difference between samples								
		Samples I and II			Samples I and III			Samples I and IV		
		t-value	d.f.	P*value	t-value	d.f.	P*value	t-value	d.f.	P* value
I	10	4.39	9	<0.002	2.56	9	<0.05	3.69	9	<0.01
II	10	6.42	9	<0.001	5.92	9	<0.001	3.92	9	<0.005
III	15	6.61	14	<0.001	8.01	14	<0.001	7.06	14	<0.001
IV	12	9.58	11	<0.001	8.06	11	<0.001	3.79	11	<0.005
V	13	11.72	12	<0.001	11.56	12	<0.001	13.45	12	<0.001

* $P < 0.05$ Significant; $P < 0.01$ Highly significant; $P > 0.05$ Not significant

d.f. = degree of freedom = n-1 where n = number of patients studied.

Table XII C

Showing statistical significance of samples when different groups were compared.

Groups which were compared	Statistical significance of samples in different comparison of groups											
	Sample I(Control)			Sample II			Sample III			Sample IV		
	t-value	d.f.	P*value	t-value	d.f.	P*value	t-value	d.f.	P*value	t-value	d.f.	P*value
I and II	1.94	18	>0.05	1.54	18	>0.1	1.74	18	>0.05	1.38	18	>0.1
III and IV	1.03	25	>0.2	0.06	25	>0.8	2.00	25	>0.05	1.27	25	>0.2
III and V	1.68	26	>0.1	0.28	26	>0.5	0.21	26	>0.8	0.69	26	>0.2
I and III	3.25	23	<0.005	1.05	23	>0.2	1.66	23	>0.1	0.30	23	>0.5
I and IV	1.92	20	>0.05	1.20	20	>0.2	0.23	20	>0.8	0.69	20	>0.2
I and V	2.75	21	<0.02	1.87	21	>0.05	1.76	21	>0.05	0.92	21	>0.2
II and III	1.34	23	>0.1	2.73	23	<0.02	4.92	23	<0.001	2.19	25	<0.05
II and IV	0.17	20	>0.8	3.25	20	<0.005	3.06	20	<0.01	0.55	20	>0.5
II and V	0.15	21	>0.8	4.69	21	<0.001	6.45	21	<0.001	4.18	21	<0.001

* P <0.05 Significant; P <0.01 Highly significant; P >0.05 Not significant.

d.f. = degree of freedom (n_1+n_2-2) where n_1 = number of patients in one group,
n_2 = number of patients in second group.

CONTROL SAMPLE

In each group sample taken just before induction of anaesthesia was termed as the control for the same patient. In Ist group mean percentage of adherence was 87.66 ± 3.41; in IInd group it was 89.94 ± 1.95; in IIIrd group it was 91.04 ± 2.12; in IVth group it was 90.12 ± 2.78 and in Vth and last group it was 90.03 ± 1.14.

GROUP I -

Granulocytic adherence of 10 patients is given in table VII. The mean granulocytic adherence of 10 cases was 87.66 ± 3.41 in first sample (control). Mean granulocytic adherence of these patients was 86.65 ± 3.86 in IInd sample. In IIIrd sample it was 85.32 ± 6.02 and in IVth sample it was 86.74 ± 3.69.

GROUP II -

Granulocytic adherence of 10 patients is given in table VIII. The mean granulocytic adherence of 10 cases was 89.94 ± 1.95 in Ist sample (control). Mean granulocytic adherence was 88.72 ± 2.24 in IInd sample and 88.34 ± 1.87 in IIIrd sample. It was 88.37 ± 1.71 in IVth sample.

GROUP III -

Table IX shows granulocytic adherence of 15 patients of this group. The mean granulocytic adherence of 15 cases was 91.04 ± 2.12 in Ist sample (control). Mean granulocytic adherence in IInd sample was 84.76 ± 4.54 and 82.14 ± 3.98 in sample IIIrd. It was 86.37 ± 2.66 in IVth sample.

GROUP IV -

Granulocytic adherence of 12 patients is given in table X. The mean granulocytic adherence of 12 cases was 90.12 ± 2.78 in Ist sample (control). It was 84.85 ± 3.33; 84.88 ± 3.37; 87.76 ± 3.32 in IInd, IIIrd and IVth sample respectively.

GROUP V -

Granulocytic adherence of 13 patients is given in table XI. The mean percentage of adherence of these 13 cases was 90.03 ± 1.14 in Ist sample (control). Mean percentage of adherence was 84.40 ± 2.25 in IInd sample. It was 82.40 ± 2.53 and 85.86 ± 1.30 in IIIrd and IVth sample respectively.

COMPARISON OF CONTROL SAMPLE WITH THE SAMPLE IInd, IIIrd and IVth.

In all the groups Ist or control sample was compared with IInd, IIIrd and IVth sample separately by applying "paired t-test" having following formula.

$$t = \frac{\bar{d} - 0}{sd/\sqrt{n}}$$

where $\bar{d}$ = difference between mean percentage of adherence of two samples which were compared.

sd = standard deviation

n = number of patients studied.

P value < 0.05 was taken as significant, P < 0.01 as highly significant and P > 0.05 as non-significant.

Table XXI B shows statistical significance between samples in different groups. When sample I and IIIrd were compared in Ist group, it showed significant difference having P value < 0.05. In all other comparisons of different groups P values were highly significant.

COMPARISON OF DIFFERENT GROUPS

To know the effect of different anaesthetic agents on granulocytic adherence, different groups were compared in different samples. For comparison of groups following formula was applied.

$$t = \frac{m_1 - m_2}{s\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where m_1 = Mean percentage of adherence in one group,

m_2 = Mean percentage of adherence in IInd group (which was compared),

n_1 = Number of patients in one group,

n_2 = Number of patients in another group.

s was calculated from the following formula.

$$s = \frac{(n_1-1)s_1 + (n_2-1)s_2}{n_1 + n_2 - 2}$$

where s_1 = Standard deviation of one group,

s_2 = Standard deviation of other group,

n_1 = Number of patients in one group,

n_2 = Number of patients in other group.

d.f. = $n_1 + n_2 - 2$

Table XII C shows the statistical significance of samples when different groups were compared. P $<$ 0.05 was taken as significant, P $<$ 0.01 highly significant and P $>$ 0.05 as non-significant. P value was non-significant when group I and II were compared. It was again non-significant when group III was compared with group IV and with group V. When group I was compared with group III, group IV and group V, P value was again non-significant. When group II was compared with group III, IV and V, P values were highly significant and significant in majority of samples except in control samples where P value was non-significant. In comparison of IInd and IVth group, sample IV was also having P value non-significant but in rest of the cases it was significant or highly significant.

DISCUSSION

DISCUSSION

It has been known for some time that a variety of anaesthetics can influence immune responses in animal and man. Speculation whether general anaesthetics decrease immunocompetence, making patients more susceptible to infections and malignancies, has been voiced (Duncan, 1976; Graham, 1911; Kanto, 1974; Lee, 1975; Logerfo, 1973; Walton, 1979).

As indicated previously, the non-specific immune response is epitomised by the inflammatory response and usually involves polymorphonuclear leukocytes as the main effector cells. The production, mobilisation and functional state of these cells all appear to be affected by anaesthesia.

With regard to productivity, there is substantial evidence starting from the early observation that prolonged exposure to nitrous oxide results in leucopaenia in human and that nearly every anaesthetic depresses white cell production. Since it is a well known fact that all anaesthetics are cellular poisons, this statement should not come

as a surprise. It seems that depressed white cell production during and following anaesthesia is due to the inhibition of cellular mitosis rather than delayed cellular maturation or release from the bone marrow. In contrast to prolonged exposure to nitrous oxide, most anaesthetic experiences in today's operating rooms are short lived and there does not appear to be any significant leucopaenia associated with them - or if there is, the inhibition of granulocyte production is reversible. Thus while it is recognised that all anaesthetics depress white cell productivity, it is likely that this depression can be withstood by healthy patients undergoing brief anaesthetic exposure (Duncan, 1976).

In most immune systems a vide variety of reactions may be affected by anaesthesia and surgery, this possibility being appreciated as early as 1875."Let us remember that chloroform does not act solely on the nerve tissue. Far from that, it has actions on all the tissues and attacks each one at a time which is a function of it's susceptibility..... Anaesthetic is not a special poison for the nervous system. It anaesthetises all the cells, benumbing all the tissues, and stoping their irritability....." (Bernard, 1875).

Phagocytosis is a primary defence mechanism against infection. Abnormalities of neutrophil function remain the most important variable of immunological defence against infections (Alexander, 1972). Since there is evidence that anaesthetic agents render cells immobile, it is conceivable that they may affect phagocytic activity and granulocyte adherence. Indeed as early as 1911, Graham showed an inhibition of phagocytosis when human and rabbit leucocytes were exposed to ether. Hamburger, in 1916, reported leucocytes in vitro after exposure to chloroform. Bruce (1967) showed that halothane anaesthesia caused a substantial reduction in the number of salmonella-bacteria ingested by each peritoneal neutrophil, 4 hours after intraperitoneal injection in mice. Kosciolek (1967) reported a decrease in phagocytosis in blood obtained from surgical patients after halothane and ether anaesthesia. Leucocytes from these patients immediately after surgery and 24 hours later, exhibited a decreased ability to phagocytose staphylococcus aureus.

Cullen, Hume, Chretien (1972) reported a decrease in phagocytosis of latex particles and nitroblue-tetrazolium (N.B.T.) reduction in patients during either halothane or nitrous oxide - narcotic

anaesthesia without surgery. In a later study (Cullen, 1974), however, halothane 0.5 - 2.5% or nitrous oxide 80% produced only minimal, statistically insignificant inhibition of latex particle phagocytosis or N.B.T. reduction. Cullen (1974) has suggested that the inhibition of phagocytosis reported in vivo during anaesthesia might result from other factors, such as stress or arterial blood flow. Recent studies have demonstrated that IgG receptor sites are present on the cell surfaces of monocytes and neutrophils, but the latter cells require complement in addition to IgG in order to accomplish efficient phagocytosis (Douglas, 1970). It might be speculated that anaesthetic agents hinder opsonization or alter the cell receptor sites.

Ostergren (1944) showed that most anaesthetic agents caused a dispersion of metaphase. Lovis and Kimball (1971) observed a similar development of C-mitosis in botanical species under influence of anaesthesia.

The possibility that anaesthetic agents may alter the course of an infection has been under consideration for the last 70 years. In 1903 Snel observed increased mortality in guinea-pigs infected with anthrax, after exposure to ether, chloroform

and chloral hydrate. Rubin (1904) made similar observations in rabbits infected with streptococci or pneumococci and exposed to ether or chloroform. Both the depth and duration of anaesthesia were important in increasing the severity of these infections. In 1910, Opie anaesthetized dogs with chloroform, with or without concomitant bacterial infection, and studied their livers. He observed that he had ".....succeeded in producing lesions of a character and intensity not obtained by simple administration of chloroform". While these early studies seem to suggest that anaesthesia may enhance an infection, other workers have observed a reverse effect. Waterhouse (1915) a surgeon from Charing Cross Hospital suggested a beneficial effect of diethyl ether in cases of pyogenic infection. Also, a bactericidal effect from ether vapour was noted by Topley (1915).

Bruce et al (1975) anaesthetised 18 patients with either halothane and nitrous oxide or nitrous oxide, thiopental-Innovar®. During anaesthesia, 7.9 less neutrophils per 100 cells contained latex and 15.7 less monocyte per 100 cells contained latex. This represent a decrease of 17 and 28 percent reduction of phagocytosis respectively from pre-anaesthetic value.

Moudgil and co-worker (1977), using in vitro exposure of polymorphonuclear leucocytes to anaesthetics including lidocaine and marcaine, demonstrated retarded chemotaxis in a dose related fashion. Stanley and associates (1978) extended these findings to in vivo exposure and were able to show that patients receiving lidocaine epidurals with epinephrine manifested depressed white cell chemotaxis. This effect was reversible after 8 hours. Inhalational anaesthetics including halothane, have also been shown to interfere with the chemotactic mechanism in humans. Hill and colleagues (1977) further demonstrated this effect in the absence of surgical stress. The effect of local anaesthetics on white cell phagocytosis are even less well studied. Lidocaine has been shown to decrease both nitroblue tetrazolium reduction and phagocytosis of latex particles by human polymorphonuclear leucocytes in vitro. The finding was only important at the site of local anaesthetic infiltration (Duncan, 1976).

By screening the above literature, it appears that majority of leucocyte functions are disturbed during and after the anaesthesia. Much work has been done on chemotaxis and phagocytosis in relation to anaesthesia but the leucocyte adherence is neglected

function till now. As we know that adherence (attachment to material) is also an important function as chemotaxis and phagocytosis; this present study was carried on 60 patients to know the effect of different anaesthetic agents on granulocytic adherence at different time intervals.

The role of granulocytes, in combating infections is undisputable, which after being attracted at the site of infection, phagocytose the infective organisms and ultimately kill the micro organism. Based on this mechanism granulocyte adherence was studied in various diseases associated with recurrent infections. Granulocyte adherence has been found to be impaired in chronic myeloid leukaemia, acute myeloid leukaemia, myelomatosis, macroglobulinemia, paroxysmal noctural haemoglobinuria (Robinowitz, 1965; Penny & Galton, 1966 and Mac Gregor et al, 1978). Lazy leucocyte syndrome and Chediak Higashi Syndrome (Boxer et al, 1974, 1976 & 1978).

The present study has been undertaken in 60 patients having different surgical procedures under different anaesthetic agents. Blood sample taken before induction of anaesthesia was taken as control sample for the same patient. Although various methods have been described for assaying the adherence

(Garvin, 1961; Brandt, 1965; Brayant and Sutcliffe, 1972; Schifter et al, 1977; Mac Gregor et al, 1974) but in the present study the method described by Klempner and Gallin (1978) has been followed, which is much simpler and is more sensitive as compared to other method available.

Blood samples from the patient were studied at same time to avoid the effect of temperature and other environmental factors (Garvin, 1961 and Kvarstein, 1969).

In the present study, the mean granulocytic adherence in control sample of group I was 87.66 $\pm$ 3.41, in group II it was 89.94 $\pm$ 1.95; in group III it was 91.04 $\pm$ 2.12; in group IV it was 90.12 $\pm$ 2.78 and in last and Vth group it was 90.03 $\pm$ 1.14. The mean G.A. obtained by Klempner and Gallin (1978) was 82.6 $\pm$ 8.2 using 80 mg of nylon fibres. The difference in adherence actively can be attributed to the different environmental factors like temperature, humidity etc. and the difference in nylon fibres used.

A highly significant ($P < 0.01$) decrease in granulocytic adherence was observed in II, III and IVth samples of the patients undergoing different surgical procedures under the influence of different

anaesthetics, except in sample III of group I where P value was significant (P $\lesssim$ 0.05).

Group I -

The mean granulocytic adherence in this group of 10 patients, who received 5% Xylocaine heavy as subarachnoid block was 87.66 $\pm$ 3.41 in control sample. 20 minutes after subarachnoid block mean granulocytic adherence was 86.65 $\pm$ 3.86. When sample I was compared to sample II, it shows highly significant (P $\lesssim$ 0.005) decrease in granulocytic adherence. In the same way, mean granulocytic adherence in III and IV sample was 85.32 $\pm$ 6.02 and 36.74 $\pm$ 3.69 respectively. Again when we compared sample I to III and sample I to IV, it leads significant (P $\lesssim$ 0.05) in I and III sample and highly significant (P $\lesssim$ 0.01) decrease in granulocytic adherence in I and IV samples comparison. By seeing these values we can say that after induction of anaesthesia there is significant decrease in granulocytic adherence at 30 minutes, after recovery from anaesthesia and even after 24 hours of surgical procedure. We can't exclude the other factors involving at this stage such as surgical stress.

Group II -

Mean granulocytic adherence of 10 patients in this group was 89.94 ± 1.95 in control sample. In II, III and IVth samples it was 88.72 ± 2.24, 88.34 ± 1.87 and 88.37 ± 1.71 respectively. When we compared sample I to sample II, sample I to sample III and sample I to sample IV, we found significant ($P < 0.05$) in I and II sample and highly significant in I and III as well as in I and IVth sample. Again this group shows continuous decrease in granulocytic adherence at different times.

Group III -

Patients of this group were given general anaesthesia having induction with thiopentone + scoline and maintained with N_2O/O_2/Flaxedil. Mean granulocytic adherence of 15 patients in control sample was 91.04 ± 2.12. Mean granulocytic adherence in sample II, III and IV was 84.76 ± 4.54, 82.14 ± 3.98 and 86.37 ± 2.66. Again control sample was compared to sample II, III and IV separately. Comparison in all samples shows highly significant ($P < 0.001$) decrease in granulocytic adherence.

Group IV -

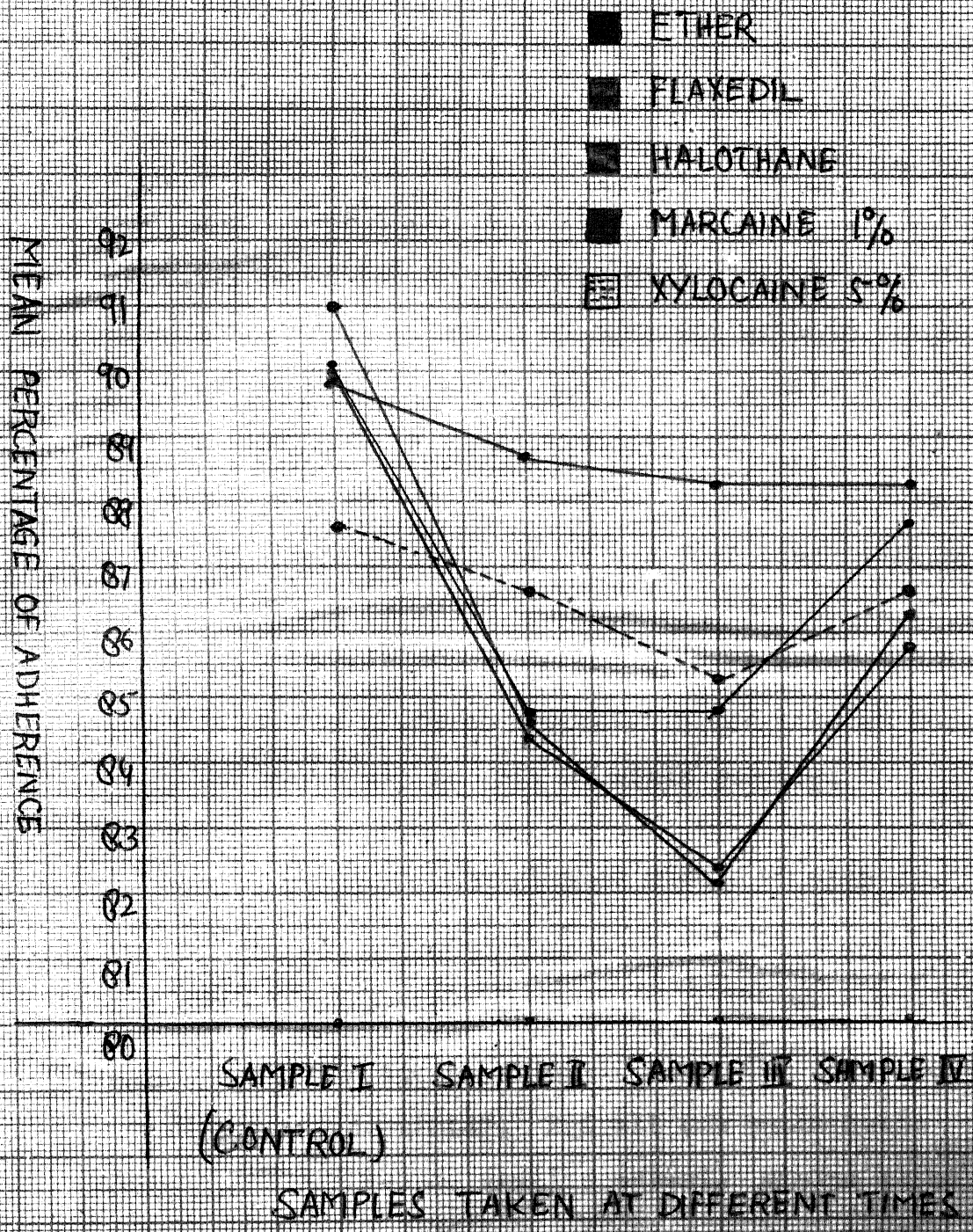
In this group patients received general anaesthesia having induction with thiopentone sodium + scoline and maintained with N_2O/O_2/Ether. Mean granulocytic adherence of 12 patients was 90.12 ± 2.78 in control sample. It was 84.85 ± 3.33, 84.88 ± 3.37 and 87.76 ± 3.32 in II, III and IV sample respectively. Comparison of control sample to sample II, III and IV separately shows highly significant ($P < 0.01$) decrease in granulocytic adherence at different times.

Group V -

These patients were induced with thiopentone sodium + scoline and maintenance was done with N_2O/O_2/Halothane. Mean granulocytic adherence of 13 patients in control sample was 90.03 ± 1.14. Mean granulocytic adherence in sample II, III and IV was 84.40 ± 2.25, 82.40 ± 2.53 and 85.86 ± 1.30 respectively. Comparison of control sample with II, III and IV sample leads significant ($P < 0.001$) decrease in granulocytic adherence at different times.

So in all the groups it was constant and significant decrease in granulocytic adherence value 30 minutes after induction of anaesthesia, at the time

GRAPH SHOWING MEAN PERCENTAGE OF ADHERENCE WITH DIFFERENT ANAESTHETICS AT DIFFERENT TIMES



of recovery from anaesthesia and after 24 hours of surgical procedure. This decrease in granulocytic adherence solely may not be due to anaesthetic agents but surgical stress may also be a contributing factor. Cullen, Hume Chretien (1972) reported a decrease in phagocytosis of latex particles and nitroblue tetrazolium (N.B.T.) reduction in patients during either halothane or nitrous oxide-narcotic anaesthesia without surgery. Decrease of granulocytic adherence in sample II may be combined effects of anaesthesia and surgery but in sample III and IV the surgical stress is almost over, so the effect may be of residual concentration of anaesthetic agents. Although hypotension is also an another factor which may affect neutrophil functions but during our study no hypotension was allowed during surgical procedure as well as after surgery.

Effect of different anaesthetic agents on granulocytic adherence

By seeing the above data, it appears that all the anaesthetic agents studied, alter the granulocytic adherence significantly. But all the anaesthetic agents are not having similar effect. Whelam et al (1982) showed significant reduction in lymphocyte number and in the response of mitogen PPD & PWM and

to histocompatibility under general but minimal changes after spinal anaesthesia. Hole (1982) further showed the same effect that depression of various lymphocyte and monocyte functions under general anaesthesia but they were absent under epidural anaesthesia. According to Hole (1982) serum cortisol increased in both pre and post operative period in general anaesthesia. Minor changes were noticed in epidural. Differences in spreading index and cytolytic activity were not seen when monocytes were cultured in medium with pooled A.B. serum, thus indicating a serum factor responsible for the monocyte depression with the patients operated under general anaesthesia.

To verify the above findings, different groups comparison were made in our study. I and II groups having spinal subarachnoid block and last 3 groups having general anaesthesia. Group I was compared to group II in different samples. The results in all the 4 samples were not statistically significant. In the same manner group I was compared to group III, IV and V but in all cases the results were statistically insignificant. Group III was compared to group IV and V to know the difference between inhalational and relevant anaesthetic agents.

but again the results in all the samples were not significant statistically.

But when group II (spinal subarachnoid block with 1% Marcaine) was compared to group III, IV and V separately, it shows statistically highly significant result with group III in all the samples except in control sample. Comparison with group IV shows significant changes in sample II and III while not significant in control sample as well as in last sample taken 24 hours after surgical procedure. Again when group II was compared to group V (Halothane) it shows statistically highly significant value in all the samples except in control samples.

The basis of adhesion is not well understood which is probably the interaction of powerful electrostatic repulsive forces between cells and substrates. There are certain divalent cations (Mn^{+2}, Mg^{+2} and Ca^{+2}) which merely neutralise the negative charges (Weiss, 1971). The other factors affecting the granulocytic adherence are interaction of chemotactic factor with phospholipid bilayer of cell membrane (Wilkinson, 1974), cyclic nucleotide (Brayant and Sutcliffe, 1973), ethanol (Brayton et al, 1970), aspirin and glucocorticoids (Dale et al, 1974 and Mac Gregor et al, 1974). Certain bacterial

products (Wilkinson, 1975) and hypophosphatemia associated with hyper alimentation (Craddock et al, 1974). The complement acts as opsonins and the interaction of C_5A and other chemotaxins with granulocytes results in a diminished negative change at the cell surface (Gallin et al, 1975). Firm contact may be mediated by surface receptors on the leucocytes that recognise sub-class IgG or C_3 complement coating the particle (Cline and Golde, 1977).

The chemotaxis, adherence and phagocytosis have all been shown to be energy dependent processes during which the glycogen content of neutrophils decreases, while oxygen consumption; lactate production, glucose utilization and hexose monophosphate shunt actively increase (Klebanoff, 1975 a). Most of this energy required is derived in the form of A.T.P. from the metabolism of glucose via glycolysis pathway. The neutrophilic membrane is permeable to glucose. Most of the enzymatic steps in glycolytic pathway are reversible. However, hexokinase, phosphofructokinase and pyruvatokinase are irreversible and of these the latter two are regulated by insulin availability (Weber et al, 1966).

From the findings of above study and the granulocytic functions observed during anaesthesia

in the past by other workers (Graham, 1911, Bruce, 1967, Kosciolek, 1967, Hume, Cullen & Chretion, 1972, Cullen, 1974; Douglas, 1970; Moudgil and co-workers, 1977), it seems that a number of vital steps in granulocytic functions are impaired in patients having anaesthetic exposure. Impairement in granulocytic functions specially the adherence activity can be held responsible for the increased frequency and susceptibility of the operated patients to various type of infections.

A careful analysis of the above data and discussion shows that all the anaesthetic agents (Lignocaine, Marcaine, Thiopentone sodium, Scoline, N_2O/O_2/Flexedil, Ether and Halothane) leads to a decrease granulocytic adherence - 20 minutes after induction of anaesthesia; at the time of recovery from anaesthesia and even 24 hours after surgical procedure.

Comparatively Marcaine given as subarachnoid block leads the less decrease in granulocytic adherence in comparison to Xylocaine given as subarachnoid block and general anaesthesia with Thiopentone sodium + Scoline + N_2O/O_2/Flexedil or N_2O/O_2/Ether or N_2O/O_2/Halothane.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Present study was conducted in 60 patients undergoing for different surgical procedures under different anaesthetic agents. The granulocytic adherence was assayed and control sample was compared to other samples taken at 30 minutes after induction of anaesthesia, at the time of recovery from anaesthesia and 24 hours after surgical procedure. To know the effect of different anaesthetic agents different groups comparison were made.

The technique described by Klempner and Gallin (1978) with minor modification was used in the laboratory for determination of granulocytic adherence.

All the patients were grouped at random into 5.

Ist group was given spinal subarachnoid block with 5% Xylocaine. In this group, mean granulocytic adherence was 87.66 $\pm$ 3.41 in control sample. In II, III and IV sample, it was 86.65 $\pm$ 3.86, 85.32 $\pm$ 6.02 and 86.74 $\pm$ 3.69 respectively. It shows generalised reduction in adherence in all the sample.

When sample I was compared to II, III & IV separately, it showed statistically significant ($P < 0.005$, $P < 0.05$, $P < 0.01$) values.

IInd group was given spinal sub-arachnoid block with 1% Marcaine. The mean percentage of adherence was 89.94 ± 1.95 and in II, III and IV sample it was 88.72 ± 2.24, 88.34 ± 1.87 and 88.37 ± 1.71 respectively. Again it shows generalised reduction in granulocytic adherence in all the samples taken at different times. Then control sample was compared to other samples in the same manner as in group I. It yielded again statistically significant values ($P < 0.05$, $P < 0.001$, $P < 0.02$).

Group III - These patients were induced with Thiopentone sodium + scoline and maintained with N_2O/O_2/Flaxedil. Mean granulocytic adherence in control sample was 91.04 ± 2.12. It was 84.76 ± 4.54, 82.12 ± 3.98 and 86.37 ± 2.66 in II, III and IV sample respectively. Again control sample was compared to II, III and IV sample separately which showed statistically highly significant ($P < 0.001$) decrease in granulocytic adherence.

Group IV - Induction was done with Thiopentone sodium + scoline and maintenance with N_2O/O_2/Ether.

Mean granulocytic adherence in control sample was 90.12 ± 2.78. It was 84.85 ± 3.33, 84.88 ± 3.37 and 87.76 ± 3.32 in II, III and IV sample. Comparison showed highly significant ($P < 0.01$) decrease in granulocytic adherence.

Group V - Received general anaesthesia having induction with Thiopentone sodium + scoline and maintenance with N_2O/O_2/Halothane. Mean percentage of granulocytic adherence was 90.03 ± 1.14 in control sample. Comparison of different samples with control sample yielded highly significant ($P < 0.001$) decrease in granulocytic adherence at different times.

For knowing the effect of different anaesthetic agents on granulocytic adherence group I was compared with group II, III, IV and V which showed insignificant value. But when group II (Marcaine) was compared with group III, IV and V separately, it showed significant difference.

The following conclusions were drawn from the present study.

1. A decrease in granulocytic adherence was found
 - 30 minutes after induction,
 - Just after recovery from anaesthesia, and
 - 24 hours after surgical procedure.

2. Decrease in granulocytic adherence was found under the influence of all the anaesthetic agents used - Xylocaine 5%,
 - Marcaine 1%,
 - Thiopentone + scoline — N_2O/O_2/Flaxedil,
 - Thiopentone + scoline — N_2O/O_2/Ether,
 - Thiopentone + scoline — N_2O/O_2/Halothane.

3. Decrease in granulocytic adherence was less when Marcaine 1% was used in subarachnoid block in comparison of general anaesthesia (Thiopentone + scoline — N_2O/O_2/Flaxedil, Thiopentone + scoline — N_2O/O_2/Ether, Thiopentone + scoline — N_2O/O_2/Halothane).

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SUMMARY

SUMMARY

Present study was conducted in 60 patients undergoing for different surgical procedures under different anaesthetic agents. The granulocytic adherence was assayed and control sample was compared to other samples taken at 30 minutes after induction of anaesthesia, at the time of recovery from anaesthesia and 24 hours after surgical procedure. To know the effect of different anaesthetic agents different groups comparison were made.

The technique described by Klempner and Gallin (1978) with minor modification was used in the laboratory for determination of granulocytic adherence.

All the patients were grouped at random into 5.

Ist group was given spinal subarachnoid block with 5% Lignocaine (Xylocaine). In this group, mean granulocytic adherence was 87.66 ± 3.41 in control sample. In II, III and IV sample, it was 86.65 ± 3.86, 85.32 ± 6.02 and 86.74 ± 3.69 respectively. It shows

generalised reduction in adherence in all the samples. When sample I was compared to II, III & IV separately, it showed statistically significant ($P < 0.005$, $P < 0.05$, $P < 0.01$) values.

IInd group was given spinal subarachnoid block with 0.5% Bupivacaine (Marcaine). The mean percentage of adherence was 89.94 ± 1.95 and in II, III and IV sample it was 88.72 ± 2.24, 88.34 ± 1.87 and 88.37 ± 1.71 respectively. Again it shows generalised reduction in granulocytic adherence in all the samples taken at different times. Then control sample was compared to other samples in the same manner as in group I. It yielded again statistically significant values ($P < 0.05$, $P < 0.001$, $P < 0.02$).

Group III - These patients were induced with Thiopentone sodium + Succinylcholine (Scoline) and maintained with N_2O/O_2/Ketamine (Flexedil). Mean granulocytic adherence in control sample was 91.04 ± 2.12. It was 84.76 ± 4.54, 82.12 ± 3.98 and 86.37 ± 2.66 in II, III and IV sample respectively. Again control sample was compared to II, III and IV sample separately which showed statistically highly significant ($P < 0.001$) decrease in granulocytic adherence.

Group IV - Induction was done with Thiopentone sodium + Succinylcholine (Scoline) and maintenance with N_2O/O_2/Ether. Mean granulocytic adherence in control sample was 90.12 ± 2.78. It was 84.85 ± 3.33, 84.88 ± 3.37 and 87.76 ± 3.32 in II, III and IV sample. Comparison should highly significant ($P < 0.01$) decrease in granulocytic adherence.

Group V - Received general anaesthesia having induction with Thiopentone sodium + Succinylcholine (Scoline) and maintenance with N_2O/O_2/Halothane. Mean percentage of granulocytic adherence was 90.03 ± 1.14 in control sample. Comparison of different samples with control sample yielded highly significant ($P < 0.001$) decrease in granulocytic adherence at different times.

For knowing the effect of different anaesthetic agents on granulocytic adherence group I was compared with group II, III, IV and V which showed insignificant value. But when group II (Bupivacaine) was compared with group III, IV and V separately, it showed significant difference.

The following conclusions were drawn from the present study.

1. A decrease in granulocytic adherence was found
 - 30 minutes after induction,
 - Just after recovery from anaesthesia, and
 - 24 hours after surgical procedure.

2. Decrease in granulocytic adherence was found under the influence of all the anaesthetic agents used - Lignocaine (Xylocaine) 5%,
 - Bupivacaine (Marcaine) 1%,
 - Thiopentone + Succinylcholine (Scoline) — N_2O/O_2/Gallamine (Flaxedil),
 - Thiopentone + Succinylcholine (Scoline) — N_2O/O_2/Ether,
 - Thiopentone + Succinylcholine (Scoline) — N_2O/O_2/Halothane.

3. Decrease in granulocytic adherence was less when Bupivacaine (Marcaine) 1% was used in subarachnoid block in comparison of general anaesthesia (Thiopentone + Succinylcholine (Scoline) — N_2O/O_2/Gallamine (Flaxedil), Thiopentone + Succinylcholine (Scoline) — N_2O/O_2/Ether; Thiopentone + Succinylcholine (Scoline) — N_2O/O_2/Halothane).
